

Clinical Course of Factor VIII Inhibitors Developed after Exposure to a Pasteurised Dutch Concentrate Compared to Classic Inhibitors in Hemophilia A

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Summary

After the introduction of a new pasteurised factor VIII concentrate (Factor VIII CPS-P) in The Netherlands in June 1990, an increase in the occurrence of inhibitors in hemophilia A patients was reported. The clinical course of this group of inhibitors ($n = 12$) was compared with hemophilia patients in whom an inhibitor developed before June 1990 (classic inhibitors) ($n = 32$). Striking differences were found between both groups not only in patient age (median 22 years versus 8 years) and number of exposure days ($<50 - >1000$ versus <50), as described in previous reports, but also in clinical course and response to treatment. In the recent group of inhibitors antibody titers showed a rapid decline when product was changed which was not the case in the group with classic inhibitors. In the group of classic inhibitors immune tolerance therapy with low dose factor VIII succeeded in 83%. Success was to a high degree dependent on the inhibitor level. In the group of recent inhibitors immune tolerance with the same concentrate was only successful in a single patient. However, once the patients were switched to another concentrate, antibody levels dropped to less than 2 BU/ml within 8 months in all patients.

It seems likely that in this group of product associated inhibitors, treatment success was due to elimination of antigen stimulation rather than induction of immune tolerance.

Introduction

The development of antibodies to factor VIII (inhibitors) is a serious complication in hemophilia treatment. It is estimated that about 5–25% of hemophilia patients develop inhibitors (1–8). In severe hemophilia inhibitors usually develop at an early age and are seldom found after 50 days of exposure to factor VIII clotting products (7). In older patients inhibitor development is rare and usually without clinical problems.

In 1991 a sudden increase in the development of inhibitors was seen in The Netherlands and Belgium (9, 10). This increase was shown to be associated with one particular factor VIII product. This pasteurised intermediate purified factor concentrate (factor VIII CPS-P) was introduced in 1990 by the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service (CLB). Inhibitors were subsequently detected in 12 Dutch patients with hemophilia A, which implied a

5-fold increased risk compared to the time period prior to the introduction of this concentrate (9). In this paper we describe the clinical course in the 12 patients who developed an inhibitor after factor VIII CPS-P administration ("recent inhibitors") and compare this to those of hemophilia patients in whom an inhibitor was detected before the new product was introduced ("classic inhibitors").

Methods

Study Population

The study population consisted of all 32 inhibitor patients registered in our center between 1966 and June 1990, as well as of 12 patients found in the national study on inhibitor formation in hemophilia patients between June 1990 and March 1992. Together they form the majority (estimated at more than 90%) of hemophilia patients with a known inhibitor in The Netherlands. Inhibitor patients could be divided into three groups.

Group 1 Classic Inhibitors

This group consisted of all inhibitor patients detected before June 1990 ($n = 32$). Nine of these patients (group 1 a) had not received immune tolerance therapy, whereas 23 patients (group 1 b) had received this treatment in a dosage of 25 U/kg body weight (bw) factor VIII, given 3 times a week on alternate days (11, 12). For immune tolerance therapy we used cryoprecipitate and a variety of concentrates.

Group 2 Recent Inhibitors

Twelve patients in whom an inhibitor was detected after the introduction of factor VIII CPS-P (9). In none of these patients antibodies had been detected before, 11 of them have severe hemophilia. Immune tolerance therapy in this group of patients varied from 25 U/kg bw factor VIII on alternate days, to 25 U/kg bw factor VIII daily.

Evaluation of Immune Tolerance Therapy

Immune tolerance therapy was considered to be successful when the inhibitor level fell to less than 2 BU/ml, with a factor VIII recovery of at least 50% of normal, a factor VIII half life of 6 h or more (13) and the absence of an anamnestic response of the inhibitor after infusion with factor VIII.

Laboratory Assay

Plasma Sampling

Plasma samples for factor VIII and inhibitor assays were drawn according to standard techniques, 4.5 ml of venous blood was withdrawn with a disposable

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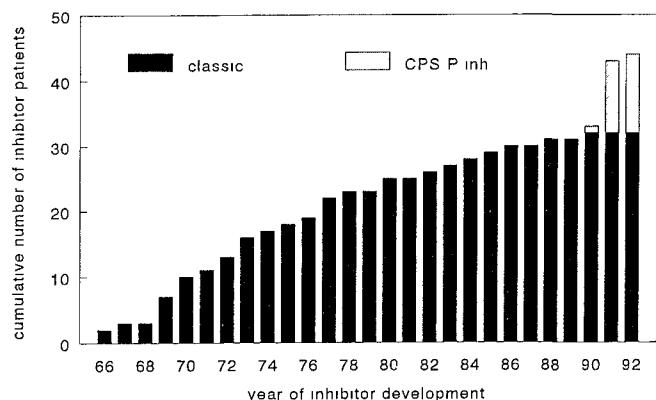


Fig 1 Cumulative number of hemophilia patients with an inhibitor detected between 1966 and 1992 in the Netherlands. Classic = classic inhibitor detected between 1966 and June 1990 (group 1). CPS-P inh = recent inhibitors, detected after the introduction of Factor VIII CPS-P in June 1990 (group 2).

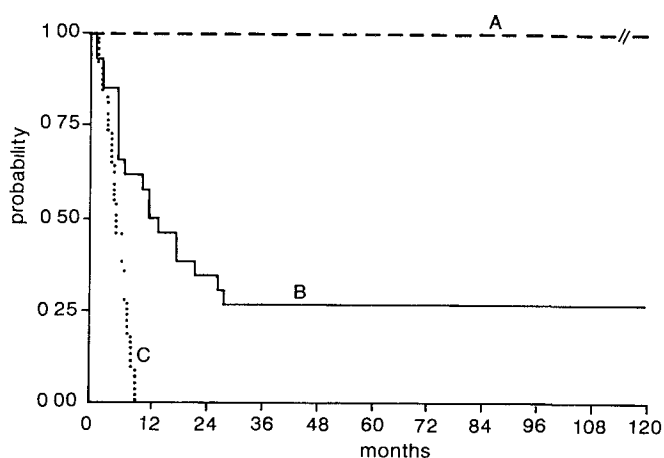


Fig 2 Disappearance of factor VIII inhibitors. A Classic inhibitor patients (n = 5) never treated with factor VIII since the detection of the inhibitor. B Classic inhibitor patients (n = 27) treated with factor VIII or with immune tolerance therapy. C Recent inhibitor patients (n = 12) who developed an inhibitor after 1990.

Table 1 General characterisation of the two groups of inhibitor patients

	classic ^a (n = 32)	recent ^a (n = 12)
age in years		
median	8	22
range	1–39	1–57
days of exposure		
<50	32	2 ¹ (2) ²
>50	0	10 ¹ (10) ²
range	12–<50	8–>1000 ¹ (8–150) ²
clinical symptoms		
yes	30	11
no	2	1
maximum inhibitor titer in BU/ml		
total group range	1–6400	5–457
successful IT ³	1–164	5–457
unsuccessful IT ³	44–450	

^a classic inhibitors detected between 1966 and 1990 (group 1)

^b recent inhibitors detected after 1990 (group 2)

¹ exposure to all factor VIII concentrates

² exposure to Factor VIII CPS-P

³ IT = Immune tolerance therapy

needle in a silicone treated Vacutainer tube in which 0.5 ml of 3.8% sodium citrate was added. Immediately after collection samples were carefully mixed and centrifuged at $3000 \times G$ for 15 min at $4^{\circ}C$, carefully pipetted off and stored in a plastic tube at $-20^{\circ}C$.

Samples of all patients with classic inhibitors and of 11 recent inhibitor patients with severe hemophilia were analysed at the coagulation laboratory of the University Hospital of Utrecht (head Dr J.W. Akkerman). Additional inhibitor measurements were performed at the local hemophilia centers.

Inhibitor Assay

Inhibitor measurements were performed using the Bethesda method as described by Kasper et al (14). In patients with positive inhibitor tests, blood samples for inhibitor measurement were taken every 4 to 8 weeks.

Factor VIII Assays

These were performed by the one stage method based on the kaoline activated partial thromboplastin time and expressed as a percentage of factor VIII present in pooled normal human plasma (15).

In Vivo Recovery

Blood samples for factor VIII assays were taken before and 15 minutes after transfusion with factor VIII. Recovery was defined as the level of factor VIII measured 15 min after infusion and the expected level calculated by the method according to Lee et al (16). Recovery was assessed every 4 to 8 weeks in all inhibitor patients who were treated with factor VIII.

Statistical Analysis

The probability of the persistence of a factor VIII inhibitor over time was evaluated by the Kaplan-Meier method (17) and the log-rank test (18). The starting-point for the life-table was the initial treatment with immune tolerance therapy, or, in non-treated patients, the time of inhibitor detection.

Results

Figure 1 shows the cumulative number of inhibitors registered in our center since 1966. Most of the gradual increase over time will reflect the increased total number of hemophilia patients and the growth of the catchment area of our center. In 1991 and 1992, however, an increased incidence of inhibitor development occurred. The clinical course of this group of inhibitor patients was compared with the classic inhibitor patient group (table 1).

1 Classic Inhibitors (group 1)

In these 32 patients the inhibitor was first detected at a median age of 8.3 years (range 1–39 years). All inhibitors were detected before the 50th day of exposure, after treatment with cryoprecipitate, or various factor VIII concentrates. Thirty patients were tested because of increased bleeding problems, two were detected at routine laboratory control.

Nine patients (group 1a) did not receive immune tolerance therapy. In five patients who did not receive any factor VIII therapy after developing an inhibitor, the inhibitor levels remained above 10 BU/ml. Three of these 5 patients died (one of an unmanageable bleeding), the other two patients still have inhibitor levels above 30 BU/ml. Four patients received only short-term factor VIII therapy (7 to 10 days), with 50 U/kg body weight daily, for life-threatening bleeding episodes or surgery. Within this period their inhibitor levels increased to over 100 BU/ml and factor VIII administration had to be discontinued.

Twenty-three patients (group 1b) received immune tolerance therapy. Nineteen patients (83%) were treated only with a low dose regimen (25 U/kg body weight 3–4 times a week), which was successful after 2–28 months in all 19 patients. In 2 patients immune tolerance was not achieved even after additional therapy with gammaglobulins and cyclophosphamide (19), and factor VIII therapy was discontinued after two years. Two other patients still receive immune tolerance therapy.

In eleven patients with inhibitor titers that never exceeded 60 BU/ml immune tolerance was obtained more easily, after 2–11 months (median 6 months) than in the eight patients with inhibitor levels above 60 BU/ml 12–28 months (median 19 months). Several factor VIII products were used, without any obvious relation between the concentrate and the success rate.

2 Recent Inhibitors (group 2)

Twelve patients in whom a persistent inhibitor developed after June 1990 were eligible for follow-up. The median age at inhibitor development was 22 years (1–57 years). Strikingly, prior to inhibitor development, most patients had received several hundreds of exposures to factor VIII with various concentrates, before they changed to Factor VIII CPS-P. After this change, it still took more than 50 exposure days to this particular concentrate before the inhibitor became apparent in ten of the twelve inhibitor patients.

In two patients factor VIII administration was discontinued, and in the other 10 patients immune tolerance therapy with factor VIII CPS-P was started, in a regimen of 25 U/kg body weight factor VIII on alternate days. This therapy was successful in only one patient, whereas inhibitor levels remained the same or even increased in nine. Dose increases to 50 U/kg body weight factor VIII (12, 20) in 4 patients with severe clinical problems, and additional corticosteroid administration (21) in one of them, did not have any beneficial effect. In all nine non-responding patients we switched to another concentrate, first in the four patients with debilitating bleedings, and later, when Factor VIII CPS-P was taken off the market (March 1992), in the other five patients. In all nine patients the change to another concentrate resulted in a rapid decrease of the inhibitor titer to less than 1 BU/ml within 8 months, normal recovery tests, and the disappearance of clinical bleeding symptoms. The concentrates that were used instead of Factor VIII CPS-P included ultra-pure monoclonally purified and intermediate purified concentrate, and pasteurised as well as SD products (Factor VIII M-SD, Hemofil M-SD, Monoclate-P, Haemate P).

In one patient immune tolerance therapy with factor VIII CPS-P proved to be successful. This patient resembled a classic inhibitor since he had developed an inhibitor within 10 exposure days at the age of 15 years.

As Fig 2 shows, inhibitors disappeared much sooner (within one year) in these recently developed inhibitor patients than in classical inhibitors ($p < 0.0001$). Moreover, success was achieved in all inhibitors.

Discussion

Previous reports have shown that the use of a particular concentrate (factor VIII CPS-P) can be associated with an increased risk of inhibitor development (9, 10). In this study we have shown that the clinical course of these inhibitors is different for classic inhibitors, with a rapid disappearance in all patients after a change of concentrate.

Usually inhibitors in hemophilia patients are detected before 50 exposure days to factor VIII (7). In our group of classic inhibitors antibodies were detected at a relatively late age, 7 years (range 1–22 years). This can be explained by the fact that most inhibitors were diagnosed before 1980 at that time replacement therapy was less frequent than today and inhibitor measurements were not performed on a routine basis. However the median age in the group of recent inhibitors was much higher 22 years (range 1–57 years). In the classic inhibitor group the total number of exposure days to any product was much lower than in the group of recent inhibitors. Moreover, the patients in this last group even had a higher number of exposure days to the new concentrate Factor VIII CPS-P prior to the inhibitor developed.

Until 1982 it was common practice to discontinue factor VIII therapy once an inhibitor had developed. In our clinic immune tolerance therapy was started in most inhibitor patients between 1982 and 1985. Successful immune tolerance therapy seems to be independent of the product used, but highly dependent on the inhibitor level (11, 12). In patients with inhibitor levels over 50 BU/ml inhibitor titers declined only slowly, whereas patients with inhibitor levels over 200 BU/ml did not respond even to the most extended and comprehensive immune tolerance regimens (19). In sharp contrast the decrease of the inhibitor titers in the group of recent inhibitors was related to the product used for immune tolerance therapy, rather than to the inhibitor level. Only an 18 months old patient with less than 10 days of exposure to factor VIII seemed to behave like a classic inhibitor. It is striking that 2 patients with inhibitor levels over 300 BU/ml showed a rapid fall of inhibitor titers after the change to another concentrate. It seems like that the mechanism of this successful treatment was not that of immune tolerance, which is thought to be the result of anti-idiotypic antibody formation (22). A possible explanation for the rapid decline of inhibitor titers is that the change of concentrate led to the removal of an unique antigenic stimulation after which the production of antibodies stopped. This also has been made plausible in laboratory investigations (23).

The cause of the antigenicity of Factor VIII CPS-P remains unclear. Its predecessor, Factor VIII CPS, was prepared by control led-pore silica adsorption (24), and initially dry heat-treated at 68°C for 72 h (CPS-HT). After the introduction of Factor VIII CPS-HT in 1988, no increase in incidence of inhibitors was seen (9). In 1990 a further improvement in virus safety was achieved with the introduction of pasteurisation (Factor VIII CPS-P) (25). Still pasteurisation itself does not seem to change immunogenicity. This may be concluded from the fact that similar inhibitor epidemics were not observed after the introduction of other pasteurised products (9) and is also supported by the fact that in 5 patients Factor VIII CPS-P was replaced by another pasteurised product with good result. Thorough analysis of the production process of Factor VIII CPS-P was undertaken but no explanation has been found. Recently, it has been shown that the pasteurised product had a much higher rate of factor Xa generation than the dry heat-treated, suggestive of the presence of small amounts of activated factor VIII (26). It is not obvious why this would lead to a higher risk of inhibitor development, and more research will be necessary to further clarify the unique event of an epidemic of inhibitors in multitransfused hemophilia patients.

In summary we conclude that the increase in the incidence of inhibitors from 1990 to 1992 in The Netherlands after introduction of Factor VIII CPS-P was followed by a rapid disappearance of all inhibitors after the patients had switched to another concentrate.

References

- Schwarzinger I, Pabinger I, Korninger C, Haschke F, Kundi M, Niessner H, Lechner K. Incidence of inhibitors in patients with severe and moderate hemophilia A treated with factor VIII concentrates. *Am J Haematol* 1987, 24: 241–5.
- Rasi V, Ikkala E. Haemophiliacs with factor VIII inhibitors in Finland: prevalence, incidence and outcome. *Br J Haematol* 1990, 76: 369–71.
- Ehrenfort S, Kreuz W, Scharrer I, Linde R, Funk M, Gungor T, Krackhardt B, Kornhuber B. Incidence of development of factor VIII and IX inhibitors in haemophiliacs. *Lancet* 1992, 339: 554–8.
- Lusher JM, Salzman PM and the Monoclate study group. Viral safety and inhibitor development associated with factor VIII C ultra-purified from plasma in hemophiliacs previously unexposed to factor VIII C concentrates. *Semin Hematol* 1990, 27, Suppl 2: 1–7.
- Sultan Y and the French hemophilia study group. Prevalence of inhibitors in a population of 3435 hemophilia patients in France. *Thromb Haemost* 1992, 67: 600–2.
- McMillan CW, Shapiro SS, Whitehurst D, Hoyer LW, Rao AV, Lazerson J, and the hemophilia study group. The natural history of factor VIII C inhibitors in patients with hemophilia A: a national cooperative study. II. Observations on the initial development of factor VIII C inhibitors. *Blood* 1988, 71: 344–8.
- Gill FM. The natural history of factor VIII inhibitors in patients with hemophilia A. *Prog Clin Biol Res* 1984, 150: 19–29.
- Peerlinck K, Rosendaal FR, Vermeylen J. Incidence of inhibitor development in a group of young hemophilia A patients treated exclusively with lyophilized cryoprecipitate. *Blood* 1993, 81: 3332–5.
- Rosendaal FR, Nieuwenhuis HK, van den Berg HM, Heijboer H, Mauser-Bunschoten EP, Van der Meer J, Smit C, Strengers PFW, Briet E and the Dutch hemophilia study group. A sudden increase in factor VIII inhibitor development in multitransfused hemophilia A patients in the Netherlands. *Blood* 1993, 81: 2180–6.
- Peerlinck K, Arnout J, Gilles JG, Saint-Remy JM, Vermeylen J. A higher than expected incidence of factor VIII inhibitors in multitransfused haemophilia A patients treated with an intermediate purity pasteurized factor VIII concentrate. *Thromb Haemost* 1993, 69: 115–8.
- van Leeuwen EF, Mauser-Bunschoten EP, van Dijken PJ, Kok AJ, Sjamsoedin-Visser EJM, Sixma JJ. Disappearance of factor VIII C antibodies in patients with haemophilia A upon frequent administration of FVIII in intermediate or low dose. *Br J Haematol* 1986, 64: 291–7.
- Mauser-Bunschoten EP, Nilsson IM, Kasper CK. Immune tolerance: A 1990 approach. In: Hemophilia and von Willebrand's disease in the 1990's, Lusher JM, Kessler CM, eds. *Excerpta Medica* 1991: 265–9.
- Over J, Sixma JJ, Doucet-De Bruine MHM, Trieschnigg AMC, Vlooswijk AA, Beeser Visser NM. Survival of 125 Iodine-labeled factor VIII in normals and patients with classic hemophilia. *J Clin Invest* 1978, 62: 223–34.
- Kasper CK, Aledort LM, Counts RB, Edson JR, Fiatantonio J, Green D, Hampton JW, Hilgartner MW, Lazerson J, Levine PH, McMillan CW, Pool JG, Shapiro SS, Schulman NR, van Eijs J. A more uniform measurement of factor VIII inhibitors. *Thromb Diath Haemorrh* 1975, 34: 869–72.
- Bouma BN, Starkenborg AE. Dilution of hemophilia plasma used as a reagent in the determination of anti-hemophilia factor A (Factor III). *Hemostasis* 1974, 3: 94–7.
- Lee ML, Gomperts ED, Kingdon HS. A note on the calculation of recovery for factor VIII infusions. *Thromb Haemost* 1993, 69: 87.
- Kaplan EL, Meier P. Non-parametric estimation from incomplete observation. *J Am Stat Assoc* 1958, 53: 457–81.
- Mantel N. Evaluation of survival data and two new rank orders statistics arising in its consideration. *Cancer Chemther Rep* 1966, 50: 163–70.
- Nilsson IM, Berntorp E, Zettervall O. Induction of immune tolerance in patients with hemophilia and antibodies to factor VIII by combined treatment with intravenous IgG, Cyclophosphamide and Factor VIII. *New Engl J Med* 1988, 318: 947–50.
- Ewing NP, Sanders NL, Dietrich SL, Kasper CK. Induction of immune tolerance to Factor VIII in hemophiliacs with inhibitors. *JAMA* 1988, 259: 65–8.
- Aznar JA, Jorquera JJ, Peiró A, García I. The importance of corticoids added to continued treatment with factor VIII concentrates in the suppression of inhibitors in haemophilia A. *Thromb Haemost* 1984, 51: 217–21.
- Sultan Y, Maisonneuve P, Kazatchkine MD, Nydegger UE. Anti-idiotypic suppression of autoantibodies to factor VIII (antihaemophilic factor) by high-dose intravenous gammaglobulin. *Lancet* 1984, 2: 765–8.
- Gilles JGG, Arnout J, Peerlinck K, Vermeylen J, Saint-Remy JMR. A sudden outbreak of inhibitors during treatment with a particular pasteurized factor VIII concentrate (FVIII-CPS-P). Characterization of specific antibodies. *Thromb Haemost* 1993, 69: 557.
- Benny AG, Scott RH, Woodfield DG. A heat-treated factor VIII concentrate prepared by controlled-pore glass adsorption chromatography. *Transfusion* 1987, 27: 174–7.
- Hiemstra H, Nieuweboer CEF, Idée MA, Claassen JE, Vos AHV, Tersmette M, Strengers PFW, Over J, Mauser-Bunschoten EP, Heijboer H, Holdrinet ACJM. Evaluation of wet pasteurization of a factor VIII concentrate produced by controlled-pore silica adsorption. *Folia Haematol* 1990, 4: 557–63.
- Barrowcliffe W, Kembell-Cook G, Tubbs JE. Inhibitor development and activated Factor VIII in concentrates. *Thromb Haemost* 1993, 70: 1065–6.

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